

Bacterial Contamination of Divers During Training Exercises in Coastal Waters

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INTRODUCTION

A common situation faced in the diving profession is the necessity for divers to operate in harbor waters that are polluted with biological and chemical substances. Biological pollutants include overtly and potentially pathogenic microorganisms. These organisms pose serious hazards to the health of divers if they are ingested or enter breaks in the skin. Infection of persons in contact with polluted water is well documented^{1, 2, 3} as are the numerous bacteria that cause these infections. We have found that *Aeromonas*, a potential pathogen emerging as a cause of wound infection⁴ and enteric disease,⁴ is prevalent in many harbor waters⁵, along with such other pathogens as *Vibrio parahaemolyticus*, *Escherichia coli*, *Klebsiella*, and *Salmonella*.⁶ Until recently, little was known about microbial hazards to divers; however, we are now assessing the extent of these hazards and the ability of various types of diving equipment to protect the divers from them. This study reports results obtained from the assessment of bacterial contamination of divers during exercises in several areas of the United States, and was conducted under the auspices of the Naval Medical Research and Development Command and the National Oceanic and Atmospheric Administration (NOAA).

Study Areas and Sampling Procedures

Harbor areas in which these studies were conducted were sites of NOAA diver operations and training exercises in Norfolk, VA, Seattle, WA, and New York, NY. In each area, water was measured (Figure 1) for temperature, dissolved oxygen concentration, transparency, and salinity. In addition, water and sediment samples were collected during the operations for determination of viable bacteria present and for identification of pathogenic bacteria that could represent a hazard to the



Figure 1. Measurement of water conditions.

divers. Bacteriologic analyses were carried out within 12-24 hr of sample collection.

Before and after each dive, the nasal passage (Figure 2a), throat (Figure 2b), ear canal (Figure 2c), and mask (nasal area, Figure 2d) of each diver were swabbed with sterile, cotton-tipped swabs. These swabs (Figure 3) were placed into 5 ml of a liquid holding medium (Cary-Blair transport broth) which maintained the bacteria in a living but nonproducing state and at ambient temperature for transport to the laboratory. In most cases, samples were transferred to appropriate culture media within 24 hr. Assessments of bacterial contamination of divers were based on an approximate quantification of viable organisms present at each body site as well as on identification of specific potential pathogens that were not present before the dive.

Water Conditions

The variations in the physical and bacteriological condi-



Figure 2a. Nasal swab.



Figure 2b. Throat swab.



Figure 2c. Ear swab.



Figure 2d. Inside of face masks.

tions of the water at the different dive sites are apparent from the data in Table 1. Water temperatures ranged from a low of 4 °C in Norfolk to 18 °C in New York Harbor at Governor's Island pier. Water temperatures in Seattle fell between these extremes. Other measured parameters of dissolved oxygen (D-O₂), transparency, and salinity varied as well, as did the number of organisms, although the latter showed only small fluctuations. Interestingly, even when the water was relatively clear (e.g. 5-8 meter transparency), organism counts were high, which illustrates the fallacy of assuming that clear

water is free of contamination by potentially harmful bacteria. This parameter can only be judged by identification and quantification of the bacteria present in the water.

Table 1. Water conditions at dive site.*

Site (date)	Temp. (°C)	D-O ₂ (ppm)†	Trans- par. (meters)†	Salinity (ppt)†	Organisms (X)
Norfolk, VA (2/80)	4	8.6	1.0	12	1
Seattle, WA					
Lake Union (7/15)	15.6	12.5	3.0	0	
Puget Sound (7/17)	9.5	9.8	5.5	29	
Puget (7/17)	11.8	11.6	5.5	28	1
Lake Union (7/22)	17.3	11.3	3.0	0	
New York, NY					
Pier (7/80)	18.0	6.4	2.0	25	1
Bight (8/80)	17.0	14.3	8.5	30	
Bight (11/80)	9.0	9.0	4.75	30	1

*All parameters measured 1 meter below surface.
 †ppm, parts per million; ppt, parts per thousand.
 ‡Direct count by epifluorescence, no. of organisms/ml.



Figure 3. Swab is placed into transport broth.



Figure 4a. Diver in wet suit.



Figure 4b. Unisuit with AGA mask.



Figure 4c. Superlight-17 hood/mask.

Diver Sampling

At the NOAA Atlantic Marine Center, Norfolk, VA, we conducted sampling procedures on 16 divers who wore four different combinations of diving gear. These were: (a) the standard neoprene foam wet suit with standard mask and backpack-supplied second stage regulator ("SCUBA"), shown in Figure 4a; (b) the Unisuit^R with AGA^R full-face mask, Figure 4b; (c) the Unisuit with the Superlight-17 hood/mask, Figure 4c; (d) the Unisuit with the Kirby-Morgan (Mark-1, Mod 0) helmet, Figure 4d. The distribution of equipment among divers participating in



Figure 4d. Kirby-Morgan (Mark-1, Mod-0).

the study is shown in Table 2. Identification of the predominant, potentially pathogenic bacteria isolated from the divers is presented in Table 3.

Table 2. Mask use by divers participating in the study.

Mask Type	Diver No. (Total No.)
SCUBA	3, 4, 5 (3)
AGA	2, 7, 9, 10, 14, 16 (6)
Superlight-17	1, 11, 12 (3)
Kirby-Morgan Mk1	6, 8, 13, 15 (4)

Table 3. Sampling of divers at the Atlantic Marine Center.

Diver No.	Mask Type	Sample site*	Organism(s) isolated
3	SCUBA	A-N	<i>Klebsiella oxytoca</i>
		A-N	<i>Aeromonas hydrophila</i>
		A-T	<i>A. hydrophila</i>
		A-E	<i>A. sobria</i>
		A-M	<i>A. sobria</i>
4†	SCUBA	B-N, A-T	<i>Enterobacter aerogenes</i>
		A-E	<i>K. pneumoniae</i>
		B-M, A-N, A-T	<i>A. sobria</i>
		A-M	<i>A. hydrophila</i>
5	SCUBA	A-E, A-M	<i>A. hydrophila</i>
9	AGA	A-E	<i>A. hydrophila</i>
		A-E	<i>A. hydrophila</i>
12‡	SL-17	B-N, A-M, A-N	<i>Ent. aerogenes</i>

*Abbreviations: A = After dive; B = Before dive; N = Nose; T = Throat; E = Ear; M = Mask.

†Divers 4 and 12 reported sore throats on the day of the dive.

‡Diver 12 was the third user of this mask.

These data give an indication of qualitative contamination of divers, i.e., the identity of bacteria that were present on the sampling sites after, and thus as a result of, the dive. Divers not included in this Table 3 (Numbers 1, 2, 6, 7, 8, 10, 13-16) showed no detectable differences in bacterial flora before and after the dive. Three of the divers who wore wet suits were contaminated after the dive by bacteria from the water, with 10 separate isolates from 12 different sites. In contrast, of the six divers in the Unisuit/AGA combination, only one showed post-dive contamination of the ear, a possible result of leakage that occurred around the face seal. The isolate of *E. aerogenes* recovered from diver no. 12 was present in the nasal passage before the dive and probably was responsible for contamination of the mask. This finding is indicative of the potential for spread of bacteria from diver to equipment and underscores the need for proper cleaning of equipment between users.

Similar sampling procedures were conducted at the NOAA Pacific Marine Center, Seattle, WA, where we followed four divers through ten days of diver training exercises in Lake Union (fresh water) and in Puget Sound (salt water). In contrast to the waters in Norfolk, Seattle area waters were relatively clear; however, counts of organisms were only slightly lower (Table 1). Assessments that represent relative bacterial contamination levels of the divers are presented in Tables 4, 5, and 6.

Table 4. Relative bacterial contamination of divers in Lake Union, 15 July.*

Diver no.	Swab Site ^b	Morning		Afternoon	
		Before	After	Before	After
1	Ear	+	4+	3+	4+
	Nose	2+	+	+	2+
	Throat	-	+	+	+
	Mask	3+	3+	4+	3+
2	E	3+	4+	+	4+
	N	4+	3+	2+	3+
	T	+	-	+	2+
	M	4+	2+	4+	3+
3	E	4+	4+	4+	4+
	N	+	2+	+	+
	T	+	+	+	-
	M	+	+	2+	+
4	E	+	4+	+	4+
	N	4+	4+	4+	2+
	T	+	2+	+	2+
	M	3+	+	2+	4+

*Based on number of colonies on primary cultures of swabs taken from the respective sites. 4+ = confluent growth; 3+ = 100 - 300 colonies, 2+ = 50 - 100 colonies, + = 1 - 50 colonies, - = no growth.

When wet suits (with hoods) were used, especially fresh water (Table 4), heavy post-dive ear contamination was common, with sporadic increase in numbers of bacteria in the nose and throat. Masks became contaminated after the first dive and remained so throughout the day. This contamination appeared to be heavier during salt water diving (Table 5), a possible reflection of the lack of fresh water with which to wash equipment between dives, and another indication of the importance of such cleaning, which was standard procedure after dock-side dives.

In most instances when divers wore the Unisuit (Table 1) bacterial levels were high, but relative levels changed little over the day's diving period. We have postulated that, while the dry suit affords excellent protection from the polluted water environment, increased humidity and heat inside the suit could promote rapid growth of indigenous bacteria. This possibility currently is under more detailed investigation.

Table 5. Relative bacterial contamination of divers in Puget Sound, 17 July.*

Diver no.	Swab Site ^b	Morning		Afternoon	
		Before	After	Before	After
1	E	4+	4+	+	4+
	N	3+	3+	4+	4+
	T	+	+	2+	3+
	M	-	4+	3+	4+
2	E	2+	4+	3+	4+
	N	2+	3+	4+	3+
	T	2+	3+	3+	3+
	M	+	4+	4+	4+
3	E	4+	4+	3+	4+
	N	3+	4+	4+	3+
	T	2+	2+	4+	4+
	M	+	4+	+	4+
4	E	+	4+	+	4+
	N	4+	4+	4+	4+
	T	3+	3+	3+	4+
	M	2+	4+	4+	4+

*Key to Table 4 applies.

In Tables 4 and 5, the data indicate that the bacterial levels in the ear of diver no. 3 consistently were high. This diver in fact developed a severe case of right and left external ear canal infection on 18 July which forced him to stop diving. On reviewing the bacterial cultures from samplings of his ears, we noticed that there were striking qualitative changes in the bacterial flora present between the morning and afternoon dives of 17 July. The flora was heavy and mixed after the morning dive, but before the afternoon dive (about 4 hours had elapsed), his ear contained a nearly-pure culture of *Pseudomonas aeruginosa*, a pathogen that commonly causes "swimmers' ear." The flora again was mixed after the dive, with the infection progressing to the point where the diver experienced discomfort and treatment became necessary. Bacteriological findings dictated that effective treatment would be acetic acid-glycerol ear drops, which rapidly cleared the infection. This case illustrates the importance of using prophylactic ear drops even when diving in apparently clear waters.

Sampling of divers in the New York Bight was done aboard the NOAA ship *George B. Kelez*, during operations in which divers were monitoring an experiment to cap the dredge spoils site with sand. The water in the Bight is relatively clean, with mild temperature, high D-O₂, and good visibility (Table 1). We again sampled the divers' ears, noses, throats, and masks, and also sites on the boot and suit. We found that the divers became contaminated by several organisms during the dive (Table 7), a probable result of suit leakage since all wore the Unisuit/AGA combination. After the dives, we attempted a disinfection procedure that involved spraying the fully-suited divers with Betadine^R Surgical Scrub

Table 6. Relative bacterial contamination of divers in Lake Union, 22 July.*

Diver no.	Swab Site	Morning†		Afternoon‡	
		Before	After	Before	After
5**	E	+	4+	*	4+
	N	4+	3+	*	3+
	T	2+	3+	*	1+
	M	3+	3+	*	3+
6**	E	3+	4+	*	3+
	N	4+	3+	*	2+
	T	+	-	*	+
	M	3+	+	*	+
7	E	+	3+		4+
	N	-	+		+
	T	-	+		-
	M	+	+		2+
8	E	3+	3+		4+
	N	3+	3+		3+
	T	3+	2+		4+
	M	-	2+		+

*Key to Table 4 applies.

**Different divers in morning and afternoon.

†Unisuit with standard mask.

‡Unisuit with AGA mask.

Solution. As shown by the data in Table 7, no organisms were recovered from the suit exterior following the Betadine spray. More detailed studies on suit disinfection currently are in progress.

Table 7. Sampling of divers during New York Bight capping operation, November 1980.

Diver No.	Sampling Site	Organisms Isolated*		
		Before Dive	After Dive	Betadine Spray†
1	Ear	—	—	ND
	Nose	—	<i>Klebsiella pneumoniae</i>	ND
			<i>Pseudomonas</i> sp.	
	Mask	—	<i>K. pneumoniae</i>	ND
			<i>Pseudomonas</i> sp.	
	Throat	—	<i>Pseudomonas</i>	ND
2	Boot	GPR	<i>Enterobacter cloacae</i>	—
	Suit	—	GNC	—
	Ear	—	—	ND
	Nose	—	—	ND
	Mask	GPR	<i>Pseudomonas</i> sp.	ND
	Throat	—	—	ND
	Boot	<i>Ent. agglomerans</i>	<i>Pseudomonas</i> sp.	—
			<i>Ent. cloacae</i>	
	Suit		<i>Pseudomonas</i> sp.	—

*Abbreviations: GPR = Gram Positive Rod; GNC = Gram Negative Coccus; ND = Not Done.

†Thorough spray with 100% Betadine Surgical Scrub solution followed by thorough rinse with fresh water.

Table 8. Disinfection of divers in Norfolk, VA, May 1981.

Disinfectant	Ranking of relative no. organisms present*								
	Suit P			Suit V			Suit A		
	Pre†	Post	Dis	Pre	Post	Dis	Pre	Post	Dis
None	10	10	10	10	10	8	2	3	6
Water	8	10	10	2	10	10	2	9	2
Betadine	9	10	5	2	6	2	2	10	3
Amway	7	10	7	5	10	3	7	7	1
Zepamine	8	10	2	1	10	2	9	9	5
Formula 100	7	10	10	6	9	2	10	10	10

*Abbreviations: Pre, pre-dive; Post, post-dive; Dis, after disinfection. Sampling site was between knee and ankle.

†Rank of 1 = 10⁴ or less CFU/10cm² suit area; rank of 10 = greater than 10⁴ CFU/10cm².

Correlations may exist between the ability of an organism to adhere to a diver or to diving equipment and its ability to initiate an infection. Our studies on *Aeromonas* show that its adherence capability is related, along with other factors, to its ability to cause human disease.⁷ Associations of this type emphasize the importance of being able to protect divers from microbial hazards present in polluted environments. Our work is progressing toward this goal by evaluation of suits that physically separate the diver from the hazard and by study of means to remove the contaminating organisms as the diver leaves the water.

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